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Clinical Role of Carbohydrate Antigen 125 in Worsening Heart Failure: a BIOSTAT-CHF study sub-analysis

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ABSTRACT

Objectives. This work aims to evaluate the association between antigen carbohydrate 125 (CA125) and the risk of 1-year clinical outcomes in patients with worsening heart failure (HF).

Background. CA125 is a widely available biomarker that is up-regulated in patients with acute HF and has been postulated as a useful marker of congestion and risk stratification.

Methods. In a large multicenter cohort of patients with worsening HF, either in-hospital or in the outpatient setting, the independent associations between CA125 and 1-year death and the composite of death/HF-readmission (adjusted for BIOSTAT risk score) were determined with the Royston-Parmar method (n=2356). In a sensitivity analysis, the prognostic implications of CA125 were also adjusted for a clinical congestion score (CCS). Data were validated in the BIOSTAT-CHF validation cohort (n=1630).

Results. Surrogates of congestion, such as NT-proBNP and a CCS, emerged as independent predictors of CA125. In multivariable survival analyses, higher CA125 was associated with an increased risk of mortality and the composite of death/HF-readmission ($p<0.001$ for both comparisons), even after adjustment for the CCS ($p<0.010$ for both comparisons). The addition of CA125 to the BIOSTAT score led to a significant risk reclassification for both outcomes (category-free net reclassification improvement=0.137, $p<0.001$ and 0.104, $p=0.003$, respectively). All outcomes were confirmed in an independent validation cohort.

Conclusions. In patients with worsening HF, higher levels of CA125 were positively associated with parameters of congestion. Furthermore, CA125 remained independently associated with a higher risk of clinical outcomes, even beyond a predefined risk model and clinical surrogates of congestion.

Keywords: CA125; antigen carbohydrate 125; Worsening Heart Failure; Congestion; Outcome.

CONDENSED ABSTRACT

We aimed to evaluate the association between carbohydrate antigen 125 (CA125) (postulated as a marker of congestion) and the risk of 1-year clinical outcomes (death and the composite of death/HF-readmission) in 2356 patients with worsening heart failure (HF). Data were validated in the BIOSTAT-CHF validation cohort (n=1630). Surrogates of congestion emerged as independent predictors of CA125. In multivariable survival analyses, higher levels of CA125 were associated with an increased risk of clinical events ($p<0.001$ for both comparisons), even beyond a predefined risk model and clinical surrogates of congestion. All outcomes were confirmed in an independent validation cohort.

ABBREVIATIONS

AHF: Acute Heart Failure

BIOSTAT-CHF: A systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure

CA125: Carbohydrate Antigen 125

eGFR: Estimated Glomerular Filtration Rate

FO: Fluid Overload

HF: Heart Failure

LVEF: Left Ventricular Ejection Fraction

NT-proBNP: N-terminal pro-brain Natriuretic Peptide

INTRODUCTION

Congestion plays a major role in the pathogenesis of acute heart failure (AHF) syndromes; however, its severity and organ distribution are largely heterogeneous (1,2). Due to the limited accuracy of routine clinical assessment for identifying and monitoring systemic congestion (1,2), there is a growing interest in searching for a reliable marker for congestion. For instance, recent evidence shows that the biologically active form of adrenomedullin (Bio-ADM) is positively associated with symptoms and signs of congestion and a higher risk of adverse outcomes in patients with worsening heart failure (HF) (3). Along this same line, plasma carbohydrate antigen 125 (CA125) has also emerged as a potential biomarker of congestion in this same setting (4,5). In fact, plasma levels of this glycoprotein are elevated in up to 60-70% of patients with AHF, and higher levels were correlated to congestion severity and adverse prognosis (4,5). Interestingly, this biomarker has also shown promising properties for monitoring the clinical course and guiding therapy following an episode of AHF (4-6). For instance, in a recent clinical trial of 380 patients with a recent episode of AHF, CA125-guided therapy was associated with a reduction of 1-year death/AHF-related risk (7). This effect was attributed to a better individualization of patients' decongestive therapy. Despite this evidence, the pathophysiology and clinical role of this glycoprotein in AHF management is not well established. Moreover, its prognostic ability for predicting clinical outcomes must be confirmed, particularly in settings where traditional prognosticators and clinical surrogates of congestion are included.

In the present work, we aimed to: a) evaluate the independent association between CA125 and the risk of 1-year clinical outcomes (death/AHF-readmission), b) determine whether the prognostic value of this biomarker remains significant after adjusting for clinical proxies of congestion, and c) relate its values with other HF-biomarkers and clinical surrogates of HF-severity/congestion.

METHODS

Study samples

BIOSTAT-CHF Cohort (Derivation cohort)

The BIOlogy Study to TAilored Treatment in Chronic Heart Failure (BIOSTAT-CHF) was a multicentre, multinational, prospective, observational study that included 2516 patients with worsening signs and/or symptoms of HF from 69 centers in 11 European countries (8). The recruitment period was 24 months, from December 2010 to December 2012. Median follow-up was 21 months [interquartile range (IQR) 15–27 months]. Patients were included after presentation with either new onset or worsening HF, which was defined as left ventricular ejection fraction (LVEF)≤40% and/or brain natriuretic peptide (BNP)>400 pg/mL or N-terminal pro-brain natriuretic peptide (NT-proBNP)>2000 pg/mL. Patients were expected and encouraged to be up-titrated to recommended treatment doses. All patients enrolled in BIOSTAT-CHF provided written informed consent to participate in the study and BIOSTAT-CHF was conducted in concordance with the declaration of Helsinki, national ethics,and legal requirements, as well as relevant EU legislation. The study was also approved by national and local ethics committees. The characteristics of the BIOSTAT-CHF cohort have been described elsewhere (8). In brief, most patients were hospitalized for AHF, and the remainder presented with worsening signs and/or symptoms of HF at outpatient clinics.

All deaths and hospitalizations were recorded. The primary outcome of interest was time to a composite of death or unscheduled hospitalization for HF. The co-primary endpoint was time to all-cause mortality. The secondary endpoint was to identify the clinical determinants of CA125.

Validation cohort

Data were validated in the BIOSTAT-CHF validation cohort consisting of 1738 HF patients from six centers in Scotland, UK, which has also been described in detail before (9). In summary, patients≥18 years old diagnosed with HF with a previous hospital admission for HF requiring diuretic treatment and current treatment with furosemide≥20 mg/day or equivalent were included. They had previously not been treated or received≤50% of target doses of angiotensin-converting enzyme inhibitors (ACEI)/angiotensin receptor blockers (ARB) and/or beta-blockers.

Congestion assessment

A composite congestion score (CCS) was calculated for individual patients at baseline using a modified and validated algorithm as described by Ambrosy et al. (10,11). The CCS was calculated by summing the individual scores for orthopnea, peripheral edema, and jugular venous distension. The presence of orthopnea and jugular venous distension contributed to the CCS with 1 point each one. However, peripheral edema used different weights to match its severity: the absence of edema, 0; edema limited to the ankles, 0.33; edema below the knees, 0.66; and edema above the knees, 1. By summing integer and non-integer scores, the final CCS was treated in the analysis as a continuous variable. Just for a sensitivity analysis, the CCS was categorized in: no congestion (CCS=0), mild congestion (CCS=1 or 2), and severe congestion (CCS≥3), by reclassifying edema into two categories: 0=no edema, edema limited to ankles, and edema below the knees, and 1=edema above the knees (11). Categorical CCS is referred heretofore as CCS_3C. Additionally, the presence of hepatomegaly and pulmonary rales >1/3 up lung fields were assessed.

CA125 measurement

CA125 was measured using the ARCHITECT CA 125 II assay (lot.81007M800), a chemiluminescent microparticle immunoassay (CMIA), on the ARCHITECT*i* System (Abbott Laboratories). The specificity and the precision of the ARCHITECT CA 125 II assay were ≤12% and 10% total CV, respectively. The sensitivity of the assay was ≤1.0 U/mL. Analytical sensitivity corresponds to the upper limit of the 95% confidence interval and represents the lowest concentration that can be distinguished from zero. The normal range of CA125 established for this assay is 35 U/mL. CA125 was measured from frozen samples. Clinical endpoint adjudications were blinded to CA125 status. NT-proBNP was measured using electrochemiluminescence on a Cobas e411 analyzer, using standard methods (Roche Diagnostics GmbH, Mannheim, Germany).

A great number of other biomarkers from multiple pathophysiological domains, including markers of inflammation, apoptosis, remodeling, myocyte stress, angiogenesis, endothelial function, and renal function were also measured (Online file 1).

Statistical analysis

Baseline characteristics among CA125 quartiles (CA125-Q4) were compared by either ANOVA, Kruskall–Wallis, or chi-squared tests, as appropriate.

Correlation heatmap and dendrogram

Spearman correlation was determined between CA125 and age, systolic blood pressure, urea, estimated glomerular filtration rate (eGFR), serum sodium, serum potassium, NT-proBNP, NYHA class, CCS, Bio-ADM, growth differentiation factor-15 (GDF-15), IL-6, ET-1, PENK, renin, and aldosterone. Because of listwise deletion, the final sample in which the correlation analysis was based included 978 patients. The same sets of variables were included in hierarchical cluster analysis (agglomerative type) with ward.D2 linkage method,

and Spearman correlation matrix as a dissimilarity measure. No significant differences were found in those included vs. excluded (Online file 2).

Association between congestion and CA125

With log-transformed CA125 as the dependent variable, the following covariates were included in the final linear regression model: CCS, age, systolic blood pressure, serum sodium, NT-proBNP, heart rate, pulmonary rales/crackles, and hepatomegaly. The added value of each covariate on model's R2 was used as an indicator of predictor's importance.

CA125 as a clinical outcome predictor

By means of a flexible parametric regression modeling –Royston-Parmar model– we determined the independent prognostic effect of CA125 on both clinical outcomes. Estimates of risk were adjusted for the outcome-specific prognostic risk score (BIOSTAT-risk score (9). The BIOSTAT risk score for mortality included age, blood urea nitrogen, NT-proBNP, serum hemoglobin, and the use of a beta-blocker. The BIOSTAT risk score for the composite endpoint included age, previous HF-hospitalization, peripheral edema, systolic blood pressure, NT-proBNP, hemoglobin, high-density lipoprotein, sodium, and use of beta-blocker.

The linearity assumption for CA125 and the prognostic risk score was tested with multivariable fractional polynomials (FP) (12). Risk estimates from the Royston-Parmar model are presented as hazard ratios (HR) with 95% CIs. Harrell C-statistics was used as the metric for the model's performance. In a sensitivity analysis, the CCS, together with BIOSTAT-risk score, was also included in the prognostic models (n=1426) to evaluate how much of its effect is mediated by clinical surrogates of congestion. The added value of CA125 on models' discriminative ability was estimated with changes in C-statistics, the

integrated discrimination improvement (IDI), and category-free net reclassification improvement (cfNRI).

We set a two-sided p-value of <0.05 as the threshold for statistical significance. Stata 15.1 (Stata Statistical Software, Release 15 [2017]; StataCorp LP, College Station, TX, USA), was used for the main analysis. Risk reclassification analyses (survIDINRI and SurvC1 modules) and dendrogram were implemented in R (Version 3.5.2; R Foundation for Statistical Computing, Vienna, Austria). A more detailed description of statistical analyses is presented in the online file 1.

RESULTS

A total of 2356 from 2516 patients included in the BIOSTAT-CHF cohort were included in this analysis. The mean age of the sample was 69±12 years; 614 (26.1%) were female, 1287 (55.6%) had a history of ischemic heart disease, and 1884 (89.3%) had an LVEF ≤40%. Median (IQR) of CA125 and NT-proBNP were 38.6 U/ml (16-125), and 2699 pg/mL (1179-5764), respectively. Baseline characteristics across quartiles of CA125 are shown in table 1. Overall, higher values of CA125 were found in older patients, those with higher rates of atrial fibrillation, worse prior NYHA class, and more severe AHF severity proxies (lower systolic and diastolic blood pressure, hemoglobin, and eGFR). Likewise, patients in the upper quartiles showed a worse echocardiographic profile (lower LVEF, higher left atrial diameter and a higher proportion of mitral valve regurgitation), and greater evidence of clinical congestion. Regarding the use of medications at baseline, lower rates of beta-blockers and ACEI/ARB were found in the upper quartiles (Table 1).

In the validation cohort (n=1630 from 1738 patients of the validation cohort), the distribution of baseline characteristics among quartiles of CA125 showed an overall higher risk profile as CA125 values increases. Higher levels of CA125 were associated with a prior history of HF hospitalizations, higher NYHA class and heart rate, poor renal dysfunction, and higher NT-proBNP. An inverse association was found between CA125 and age, systolic blood pressure, hemoglobin, sodium, and potassium (Online file 3).

Heatmap and dendrogram

Figure 1a shows the Spearman correlation heatmap coefficients. CA125 had the strongest correlation with NT-proBNP (rho=0.42, p<0.001), followed by GDF-15 (rho=0.40, p<0.001), ET-1 (rho=0.38, p<0.001), IL-6 (rho=0.35, p<0.001), Bio-ADM (rho=0.35,

p<0.001), the CCS (rho=0.34, p<0.001), NYHA class (rho=0.26, p<0.001), heart rate (rho=0.23, p<0.001), and serum sodium (rho=-0.15, p<0.001).

In a hierarchical cluster analysis (Figure 1b), CA125 clustered with the combined effect of Bio-ADM, ET-1, IL-6, NT-proBNP, and GDF-15, which, in turn, clustered together with the combined effect of NYHA class, and the CCS.

Similar findings were obtained in the validation cohort (Online file 4). CA125 correlated with NT-proBNP (rho=0.52, p<0.001), GDF-15 (rho=0.37, p<0.001), Bio-ADM (rho=0.30, p<0.001), NYHA class (rho=0.24, p<0.001), PENK (rho=0.20, p<0.001) heart rate (rho=0.20, p<0.001), serum potassium (rho=-0.16, p<0.001), systolic blood pressure (rho=-0.15, p<0.001) , urea (rho=0.15, p<0.001), eGFR (rho=-0.13, p<0.001), and serum sodium (rho=-0.13, p<0.001). The dendogram showed that CA125 clustered with NT-proBNP. Both clustered together with the combined effect of NYHA class, Bio-ADM, and GDF-15 (Online file 5).

CA125 as marker of congestion

The analysis showed that the most important predictors of log transformed CA125 (line-up based on the magnitude of its contribution to the total R^2 of the model) were: 1) NT-proBNP [ΔR^2=0.184; p<0.001], CCS [ΔR^2=0.040; p<0.001], age [ΔR^2=0.020; p<0.001], serum sodium [ΔR^2=0.015; p<0.001], heart rate [ΔR^2=0.006; p=0.001], pulmonary rales/crackles [ΔR^2=0.006; p=0.004], systolic blood pressure [ΔR^2=0.003; p=0.027], and hepatomegaly [ΔR^2=0.0008; p=0.226]. The full model R^2 was 0.277. Detailed model is shown in Table 2. The contribution of adding the block of all congestion-related variables (NT-proBNP, CCS, serum sodium, pulmonary rales/crackles and hepatomegaly) to age, heart rate and systolic blood pressure represents a ΔR^2=0.22; p<0.001, which is equivalent to

79.4% of the total model R^2. Figure 2 shows the independent association between CCS and NT-proBNP with log transformed CA125.

In the validation cohort, the independent variables associated with log transformed CA125 were, in order of importance: NT-proBNP [ΔR^2=0.256; p<0.001], serum potassium [ΔR^2=0.016; p<0.001], serum sodium [ΔR^2=0.015; p<0.001], heart rate [ΔR^2=0.014; p<0.001], systolic blood pressure [ΔR^2=0.005; p=0.004], age [ΔR^2=0.003; p=0.033], and prior admission for AHF [ΔR^2=0.002; p=0.015]. The full model R^2 was 0.313.

Clinical endpoints

Derivation cohort

Mortality

During a 1-year follow-up, 369 deaths were registered. Unadjusted mortality rates (per 100 person-years) significantly differed across quartiles of CA125 (8.53, 12.17, 21.21, and 29.33 for Q1, Q2, Q3, and Q4, respectively; p<0.001). Kaplan-Meier curves are shown in online file 6.

In a multivariable survival analysis that included the BIOSTAT risk score for mortality as a covariate, the continuum of CA125 revealed a positive, sigmoid-shaped association with the risk of mortality (C-statistics=0.757; overall p-value<0.001) (Figure 3a). The adjusted association between CA125 (log CA125 and quartiles) and mortality is presented in table 3.

Composite of death and/or rehospitalization for AHF

At a median follow-up of 1-year, 678 combined endpoints were ascertained. The rates (per 100 person-years) of this composite endpoint significantly differed among CA125

quartiles (18.6, 30.3, 41.7, and 56.6 for Q1, Q2, Q3, and Q4, respectively; p<0.001). Kaplan-Meier curves are shown in online file 7.

Multivariable analysis showed that CA125 was significantly associated with this endpoint, through a positive and also sigmoid-shaped curve (C-statistics=0.719; overall p-value<0.001) (Figure 3b). Risk estimates (log CA125 and quartiles) for the composite endpoint are presented in table 3. In the derivation cohort, CA125 (log CA125 and quartiles) remained significantly associated with the risk of the combined endpoint (Table 3).

Sensitivity analysis

In a sensitivity analysis, where the CCS was added as an additional covariate (n=1426), the results were in line with the main findings (Figures 4a and 4b). Furthermore, we also found that the predictive ability for all-cause mortality of CA125 for both endpoints (here dichotomized at 35 U/mL) was consistent across all levels of the CCS_3C (Online files 8 and 9).

Survival model's performance

<u>All-cause mortality.</u>

Online file 10 summarizes the survival model´s performance. The models that included CA125 showed a significant risk reclassification compared to BIOSTAT risk score (IDI=0.008, 95% CI: 0.001-0.020, p=0.010 and cfNRI=0.137, 95% CI: 0.073-0.184, p<0.001) or BIOSTAT risk score+NT-proBNP (IDI=0.005, 95% CI: 0.001-0.013, p=0.043 and cfNRI=0.106, 95% CI: 0.021-0.161, p=0.027). However, the ΔC statistic did not significantly differ when adding CA125 to BIOSTAT risk score (Δ=0.004, CI:-0.001-0.010) or BIOSTAT+NT-proBNP (Δ=0.001, CI:-0.002-0.004).

Composite of death and/or rehospitalization for AHF

The addition of CA125 led to a significant risk reclassification compared to BIOSTAT risk score (IDI=0.009, 95% CI: 0.003-0.017, p<0.001 and cfNRI=0.104, 95% CI: 0.061-0.150, p=0.003) and borderline when compared to BIOSTAT risk score+NT-proBNP (IDI=0.004, 95% CI:0.001-0.010, p=0.040 and cfNRI=0.050, 95% CI:-0.013-0.086 p=0.106). The ΔC statistic was significantly higher when compared to BIOSTAT risk score (Δ=0.006, 95% CI: 0.001-0.010) but not when compared to BIOSTAT risk score+NT-proBNP (Δ=0.002, CI: -0.001-0.005).

Validation cohort

In a univariate as well as in multivariable context, CA125 also demonstrated to be a significant and independent predictor for 1-year all-cause mortality and for the composite of mortality and/or AHF-readmission (Table 3).

DISCUSSION

The present study confirms the role of CA125 as a surrogate of congestion and its utility as a prognostic biomarker in worsening HF, confirming findings from previous smaller studies. In fact, the present data from a sub-analysis of the BIOSTAT-CHF and the validation cohorts indicate that CA125 was strongly associated with higher risk of 1-year all-cause mortality and the combined of all-cause death and hospitalization for HF. The merit of this study stems from a rigorous model's adjustment including clinical surrogates of systemic congestion. Moreover, through a cluster analysis, CA125 grouped with recognized biomarkers of congestion and inflammation. Congruent results were also found in the validation cohort.

CA125 as a marker of congestion

Symptoms and signs of congestion are found in most patients with AHF; however, its presence and severity are largely heterogeneous (1,2). Unfortunately, traditional clinical assessment of congestion through symptoms and signs has shown a limited accuracy (1,2). Thus, current experts recommend an integrative multi-parameter-based evaluation of congestion using clinical assessment, biomarkers, and supplemented with technical assessments (1-3). Despite these recommendations, a well-validated tool for clinical assessment of congestion is still an unmet need. In the last years, CA125 has emerged as a novel useful biomarker of congestion (4,5). The pathophysiology of CA125 in AHF remains largely unknown. What is known is that this large glycoprotein is synthesized by mesothelial cells in response to an increase in hydrostatic pressures and/or inflammatory mediators (4,5). In patients with AHF, CA125 has shown to be related to symptoms or signs of fluid overload, such as peripheral edema, serosal effusion, pulmonary wedge pressure, and increasing cardiac pressures (4,5,13,14). Consistent with this notion, a recent meta-analysis showed that

patients with significant serosal effusions had higher CA125 levels (15). However, in patients with refractory congestive HF treated with continuous peritoneal dialysis, CA125 decreased in parallel with decongestion and despite the peritoneal irritation induced by the presence of an osmotic solution into the peritoneum (16). These findings suggest that increases in CA125 associated with serosal effusion are parallel processes caused by common pathophysiological mechanisms, and not necessarily a cause-effect phenomenon. Interestingly, in the present study, we found that CA125 was strongly associated with well-validated proxies of clinical congestion. In addition, their values were also positively related to biochemical surrogates of increased filling pressures/congestion such as NT-proBNP and Bio-ADM (3). In a recent analysis of 2179 patients with worsening HF, ter Maaten et al. reported Bio-ADM was related to symptoms and signs of congestion (3). Interestingly, in the cluster analysis CA125 and Bio-ADM grouped together. These findings suggest both biomarkers may play a crucial role in identifying the degree of congestion in patients with worsening HF.

Future studies should investigate the exact role of CA125 in the identification of intravascular vs. extravascular congestion. In addition, a formal comparison with other novel biomarkers of congestion is still required.

CA125 and risk stratification

Prior studies have reported a significant association between CA125 and the risk of death and HF-readmission in different AHF scenarios (4-6,13-15,17). This is the largest study in AHF confirming the prognostic value of CA125 independent of standard prognosticators. Additionally, present findings also support that the value of these biomarkers for risk stratification is independent of traditional symptoms/signs of congestion. Interestingly, the association between CA125 and adverse clinical events remained significant in those with

mild, moderate, and severe congestion. Such behavior opens an avenue for its incorporation in multi-parametric congestion scores.

CA125 assessment in AHF. Ready for clinical implementation?

In our opinion, the following aspects may contribute to endorsing the use of CA125 into a routine clinical practice: a) the lack of standardized biomarkers for assessing congestion in daily clinical practice; b) wide availability and low cost (≈1€), c) independent predictive ability beyond clinical and biochemical markers of congestion (including natriuretic peptides) (4-6,17); d) longitudinal trajectories highly associated with risk of adverse outcomes (4-6), and; e) potential utility for guiding depletive therapy (7,18). Regarding, this last point, the CHANCE-HF trial, performed in 380 patients discharged for AHF and high CA125, CA125-guided therapy (up or down-titrating depletive treatment when CA125 increased or decreased during follow-up) was superior to the standard of care in terms of reducing the risk of the composite of 1-year death or AHF readmissions (7). More recently, a CA125-guided diuretic strategy (intensive diuretic therapy in patients with CA125>35 U/ml and more conservative approach when CA125≤35 U/ml) was evaluated in an open-label randomized study that enrolled 160 patients with AHF and renal dysfunction at presentation (mean eGFR of 33.7±11.3 mL/min/1.73m^2) (18). In this trial, the CA125-guided strategy significantly improved eGFR and other renal function parameters at 72h (18). In summary, and beyond logistic issues, there is promising data supporting the clinical utility of CA125 as HF biomarker, not only as a spot prognosticator but also for monitoring the course of the disease/congestion status and guiding depletive therapy. Lastly, and along this line, given the long half-life of CA125 (7-12 days) (4,5), it can be measured during early decompensation without noticing significant changes (17). Conversely, we have found

meaningful and significant changes at 30 days and further follow-up, being these changes strongly associated with prognosis (4-6).

Limitations

Some limitations need to be addressed. First, these results do not apply to patients with stable chronic HF. Second, the lack of assessment of echocardiographic parameters of right ventricular failure and a more detailed evaluation of symptoms and signs of congestion precludes evaluating the contribution of these parameters with CA125. Third, we couldn't validate in the external cohort (BIOSTAT-validation cohort) the regression formula obtained in the main cohort given the lack of availability of several biomarkers and congestion parameters. Instead, a similar statistical approach followed on the main cohort was applied to the validation cohort, using the set of covariates available. Fourth, the clinical utility of this biomarker in underrepresented AHF phenotypes - such as heart failure with preserved ejection fraction, and patients with greater renal dysfunction - remains to be determined. Fifth, we cannot evaluate the influence of new treatments, such as sacubitril/valsartan, on the magnitude or direction of these findings. Sixth, since this is a predominant cohort of Caucasian patients, these findings cannot be extrapolated to other races. Finally, we have not compared fresh samples to frozen samples and therefore cannot exclude storage/freeze/thaw artifacts.

CONCLUSION

In patients with signs and/or symptoms of worsening HF, circulating levels of CA125 were independently and positively associated with clinical surrogates of congestion. Their levels were also highly predictive of 1-year all-cause mortality and of the composite of mortality and HF-hospitalization. In addition, such prognostic effect showed to be independent of the

severity of systemic congestion. Further studies are warranted to confirm the role of CA125 as a marker of congestion and unravel the role of this glycoprotein in the pathophysiology of AHF syndromes.

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FIGURE LEGENDS

Figure 1. Heatmap and dendrogram

1a. Biomarker position of CA125 in a correlation heatmap. Correlations are based on Spearman's rho as a correlation coefficient.

1b. Biomarker position of CA125 in hierarchical cluster analysis.

Bio-ADM: Bio-adrenomodullin; CA125: antigen carbohydrate 125; CCS: clinical congestion score; eGFR: estimated glomerular filtration rate; ET-1; endothelin-1, GDF-15: growth differentiation factor-15; IL-6: interleukin-6; NT-proBNP: N-terminal pro-brain natriuretic peptide; NYHA: New York Heart Association; SBP: systolic blood pressure.

N=978 patients.

Figure 2. CCS and NT-proBNP as predictors of logarithm transformed CA125.

2a. CCS and logarithm transformed CA125.

2b. NT-proBNP and logarithm transformed CA125.

Covariates used for adjustment: age (years), systolic blood pressure (mmHg), heart rate (bpm), serum sodium (mmol/L), NT-proBNP (pg/mL), hepatomegaly, pulmonary rales, and the CCS.

N=1268, model R^2=0.277

CA125: antigen carbohydrate 125; CCS: clinical congestion score**;** NT-proBNP: N-terminal pro brain natriuretic peptide.

Figure 3: A 1-year multivariable analysis.

3a. All-cause mortality

3b. Mortality/HF-readmission

The analysis adjusted by the BIOSTAT risk score for mortality (N=2356). HR depiction along the continuum of CA125 (35 U/ml as reference).

HF: heart failure; HR: hazard ratio; CA125: carbohydrate antigen 125.

Figure 4: A 1-year multivariable analyses including the CCS.

4a All-cause mortality

4b. Mortality/HF-readmission

The analysis adjusted by the 1-y BIOSTAT risk score for the composite endpoint and the CCS (N=1426). HR depiction along the continuum of CA125 (35 U/ml as reference).

HF: heart failure; HR: hazard ratio; CA125: carbohydrate antigen 125; CCS: clinical congestion score.

CLINICAL PERSPECTIVES

The present study confirms the role of CA125 as a surrogate of congestion and its utility as a prognostic biomarker in patients with worsening HF. The present data from a sub-analysis of the BIOSTAT-CHF and the validation cohorts indicate that CA125 was strongly associated with a higher risk of 1-year all-cause mortality and the combined event of all-cause death and hospitalization for HF. Moreover, through a cluster analysis, CA125 grouped with recognized biomarkers of congestion and inflammation.

TRANSLATIONAL OUTLOOK

Measurement of CA125 constitutes a valuable tool both for estimating the degree of congestion in worsening HF, which can be very useful for clinical management, as well as for risk stratification. The fact that it is a biomarker widely available, cheap and with standardized measurements, favor its routine use in clinical practice.